Suppression of Histone Deacetylation in Vivo and in Vitro by Sodium Butyrate*

(Received for publication, February 16, 1978)

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SUMMARY

In HeLa cells which have been exposed to 5 mM sodium butyrate for 21 h, the level of histone acetylation is greatly increased as compared to control cells (Riggs, M. G., Whittaker, R. G., Neumann, J. R., and Ingram, V. R. (1977) Nature 268, 462-464). Our experiments indicate that the increase in the relative amounts of multiacetylated forms of histones H4 and H3 following butyrate treatment is the result of an inhibition of histone deacetylase activity.

Since the original observation that histone acetylation represents a postsynthetic modification of the polypeptide chain (1, 2) which is reversible (3, 4), the biological function of the reaction has aroused considerable interest. There is much evidence to support the hypothesis that acetylation provides an enzymatic mechanism for modulating the interactions between histone and DNA (1, 2) and that increased acetylation, by releasing constraints upon the DNA template, alters the structure and function of chromatin (for recent reviews, see Refs. 5 and 6). A direct test of this hypothesis became possible when it was observed that in vivo levels of histone acetylation could be increased experimentally by exposing HeLa cells to 5 mM sodium butyrate (7). We have shown that, as a consequence of increased histone acetylation, the associated DNA sequences became more accessible to attack by DNase I, but not by staphylococcal nuclease (8). In HeLa cells and in avian erythrocytes, the multiacetylated forms of histone H4 and H3 are preferentially released during limited digestions with DNase (8). Since it has been repeatedly observed that limited DNase I digestions preferentially degrade the transcriptionally active DNA sequences in avian erythrocytes (9) and other cell types (10-13), the association of acetylated histones with active genes is confirmed.

The postsynthetic acetylation of histones involves a transfer of acetyl groups from acetyl coenzyme A (2, 14) to the e-amino groups (15, 16) of specific lysine residues in the NH2-terminal regions of the polypeptide chain (17-21). Histones H3 and H4, which play a key role in the organization of the nucleosome (22-24), each have four sites of internal acetylation, and in most cell types, each of these histones comprises a mixture of polypeptide chains containing 0 to 4 e-N-acetyllysine residues. The various modified forms are separable by high resolution gel electrophoretic techniques (25-27). Acetate uptake into histones in vivo is known to be reversible, and the overall level of acetylation involves an equilibrium between acetate uptake, as catalyzed by acetyltransferases, and acetate removal, as catalyzed by histone deacetylases. The effect of butyrate on both these processes has now been studied in intact HeLa cells, and the observed inhibition of histone deacetylation has been confirmed by studies of butyrate inhibition of the purified histone deacetylase from calf thymus nuclei.

MATERIALS AND METHODS

HeLa S-3 cells were grown in Eagle's minimal essential medium supplemented with 5% fetal calf serum in the presence or absence of 7 mM sodium butyrate as described by Riggs et al. (7). Nuclei were isolated as described previously (38). Histones were extracted from the purified nuclei in 0.4 N H2SO4, and precipitated in 10 volumes of ethanol. The histones were analyzed by high resolution electrophoresis in 23-cm long, 12% polyacrylamide gels containing acetic acid and urea (27).

For studies of radioactive acetate uptake, equal aliquots (350 ml) of HeLa cell suspensions containing 5 to 6 x 105 cells/ml were incubated with either 1.5 mCi of [methyl-3H]acetate (specific activity, 700 mCi/mmol) in ME medium, or with 300 mCi of [1-14C]acetate (specific activity, 4 mCi/mmol) in ME medium containing 7 mM sodium butyrate. After 5-, 10-, and 15-min incubations at 37°C, 100-mg aliquots of each suspension were mixed and diluted with an equal volume of ice-cold ME medium. Cells were collected by centrifugation at 2000 x g for 10 min, and nuclei were isolated. Histones were extracted in 0.4 N H2SO4, precipitated in acetone, and redissolved at a concentration of 1 mg/ml of 0.9 M acetic acid, 9 M urea, 1% 2-mercaptoethanol. Aliquots were taken for measurement of histone H3 and H4 activities and for electrophoretic analysis (27). The gels were stained with 0.1% Amido black in 35% methanol, 15% acetic acid, and the densitometric tracings of the stained bands were recorded. The bands corresponding to individual histone fractions were cut out, and the radioactivity in each band was determined by combusting the gel slices in a Packard Sample Oxidizer and measuring the H2O and CO2 activities of the separated combustion products.

In the acetate "turnover" experiments, equal aliquots (500 ml) of a HeLa cell suspension containing 5 to 6 x 105 cells/ml were incubated for 15 min at 37°C in ME medium containing either 2.5 mCi of [methyl-3H]acetate or 0.5 mCi of [1-14C]acetate. The H2-labeled cells were harvested, washed twice with warm, nonradioactive ME medium and resuspended in ME medium containing 7 mM sodium butyrate. Both suspensions were incubated at 37°C, withdrawing samples at 0, 10, 20, 30, and 60 min. The H2-labeled (control) cells and H3-labeled (butyrate-treated) cells were mixed prior to isolation of the nuclei and extraction of histones. The [H3- and [14C]acetyl content of the histone samples was determined as described.

For studies of the effects of butyrate on histone deacetylation, the enzyme was prepared from calf thymus nuclei as previously described (29) and tested on purified H4 histone which had been labeled in situ with [H3]acetate to a specific activity of 10,200 cpm/mg. The same preparation of the histone deacetylase was used in all experiments, and the kinetics of [H3]acetate release in the presence or absence of sodium butyrate were studied as follows. Equal volumes of enzyme solution and histone solution (1 mg of H4/ ml of 0.14 M NaCl, 25 mM Tris-HCl, pH 7.3, containing the indicated concentrations of sodium butyrate) were mixed and incubated at...
It has been observed previously that prolonged exposure of HeLa cells to sodium butyrate leads to an accumulation of multiacetylated forms of histones H3 and H4 (7), and that the altered extent of histone acetylation is also evident in isolated nucleosomes (8). The DNA sequences associated with multiacetylated histones are highly susceptible to attack by DNase I (8), and thus it appears that acetylation can influence chromatin structure at the nucleosomal level. The rate of acetate uptake into histones H3 and H4 is known to increase at times of gene activation (5, 8) whereas deacetylation is suppressed (4-6), and it follows that the balance between acetylation and deacetylation is subject to physiological control. How does butyrate affect that balance to increase the proportions of the multiacetylated histone forms in HeLa cells?

We have compared the effects of 7 mM butyrate upon the incorporation and release of radioactive acetate in four histone classes, H1, H2b, H3, and H4. In the uptake experiments, control cells were incubated in the presence of [3H]acetate, while butyrate treated cells were incubated with [14C]acetate. Aliquots of the two cell cultures were withdrawn at 5-min intervals and mixed prior to isolation of the nuclei and extraction of the histones. After electrophoresis of the latter in acid/urea-polyacrylamide gels, the histone bands were analyzed for 14C and 3H activities and the ratio of 14C to 3H was determined at each point. The rates of acetate incorporation into histones H2b, H3, and H4 were essentially the same in control and butyrate-treated cells, and the corresponding 14C/3H ratios remained virtually constant (Fig. 1). (Acetate uptake into histone fraction H1 is only 10% or less of that seen in H4 and is due, not to lysine acetylation, but to acetylation of the NH₂-terminal serine residues, a reaction which takes place in the cytoplasm at the time of histone synthesis (30).)

The invariant 14C/3H ratio in this case indicates that butyrate has no detectable influence on H1 synthesis during the time interval studied.) The incorporation of [3H]acetate in the NH₂-terminal portion of newly synthesized H4 molecules occurs also at the time of H4 synthesis and it is irreversible. Only a small part of the radioactive acetate present in HeLa H4 represents substitution at the NH₂ terminus, as judged by the corresponding labeling of histone H1. This reflects limited synthesis of H4 during the 15-min "pulse." One would expect that the presence of a stable NH₂-terminal acetyl group on histone H4, but not on histones H2b and H3, would differentially influence the slopes of plots of 14C/3H versus time for the different histones during the "cold chase."

Butyrate has a marked inhibitory effect on the turnover of previously incorporated acetyl groups in HeLa cell histones, as shown by the experiments summarized in Fig. 2. In these experiments, control cells containing [3H]acetylated histones were incubated in nonradioactive, butyrate-free medium, while cells containing [14C]acetylated histones were incubated in nonradioactive medium containing 7 mM sodium butyrate. Samples were withdrawn at the indicated times, and the [14C]-labeled cells were mixed with the [3H]-labeled cells prior to isolation of the nuclei and extraction of the histones. After electrophoretic separation of histones H1, H2b, H3, and H4, the ratio of 14C to 3H was determined for each histone at each time point. The progressive increase in this ratio for the nucleosomal histones H2b, H3, and H4 (Fig. 2) indicates that the [3H]acetate content of the control cell histones declines rapidly relative to the [14C] content of the corresponding histones in the butyrate-treated cells. The contrast between the rates of acetate release in control and butyrate-treated cells is illustrated by the decrease in the specific activities of the total histone fraction after a 1-h cold chase: from 5710 cpm/mg to 2420 cpm/mg (a decrease of 58%) in the control cells and from 6150 cpm/mg to 5200 cpm/mg (a decrease of only 16%) in the butyrate-treated cells. (No change in the 14C/3H ratio was seen for histone H1, in accord with the view that acetate incorporation at the NH₂-terminal serine residue of H1 is essentially irreversible (30).)

The results show that butyrate suppresses the deacetylation of histones in HeLa cells. Since it has no appreciable effect on the uptake of acetate (Fig. 1), the net effect is a progressive accumulation of the acetylated forms of histones.
histones H3 and H4, as previously observed in isolated nucleosomes (8) and in whole nuclei (7) of HeLa cells exposed to butyrate for 21 h.

To confirm the view that butyrate suppresses the enzymatic deacetylation of histones, we have tested the effects of increasing concentrations of sodium butyrate on the kinetics of [3H]acetate release from calf thymus nuclei (29). The results are summarized in Fig. 3 which shows that very low concentrations of sodium butyrate effectively suppress deacetylase activity. Judging by the extent of [3H]acetate release at 30 min, 5 mM butyrate suppresses deacetylation of histone H4 by about 84%. It is not known whether butyrate uniformly inhibits the deacetylation of different lysine residues in the NH₂-terminal region of the polypeptide chain or if the kinetics of [3H]acetate release under these conditions are the same for mono- and multiacetylated forms of H4. These questions are now under investigation.

Finally, it is clear from these and earlier studies (20, 29, 31, 32) that the activity of the histone deacetylases is subject to control. The activity of hepatic deacetylases immediately increases as RNA synthesis is inhibited by the hepatocarcinogen, aflatoxin B₁ (31). How such regulation is achieved is not presently known, but the apparent loss of an inhibitor during purification of the deacetylase from calf thymus nuclei (29) suggests that regulatory factors directly influence deacetylase activity within the cell nucleus and thus influence the structure of the nucleosomes.
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